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- ☒ 1: Wound Repair Regen. 2003 Jan-Feb;11(1):35-45.

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Long-term remodeling of a bilayered living human skin equivalent (Apligraf) grafted onto nude mice: immunolocalization of human cells and characterization of extracellular matrix.

Guerret S, Govignon E, Hartmann DJ, Ronfard V.

Biomaterials Laboratory, University C. Bernard, Lyon, France.

Type I collagen is a clinically approved biomaterial largely used in tissue engineering. It acts as a regenerative template in which the implanted collagen is progressively degraded and replaced by new cell-synthesized tissue. Apligraf, a bioengineered living skin, is composed of a bovine collagen lattice containing living human fibroblasts overlaid with a fully differentiated epithelium made of human keratinocytes. To investigate its progressive remodeling, athymic mice were grafted and the cellular and the extracellular matrix components were studied from 0 to 365 days after grafting. Biopsies were analyzed using immunohistochemistry with species-specific antibodies and electron microscopy techniques. We observed that this bioengineered tissue provided living and bioactive cells to the wound site up to 1 year after grafting. The graft was rapidly incorporated within the host tissue and the bovine collagen present in the graft was progressively replaced by human and mouse collagens. A normal healing process was observed, i.e., type III collagen appeared transiently with type I collagen, the major collagen isoform present at later stages. New molecules, such as elastin, were produced by the living human cells contained within the graft. This animal model combined with species-specific immunohistochemistry tools is thus very useful for studying long-term tissue remodeling of bioengineered living tissues.

PMID: 12581425 [PubMed - indexed for MEDLINE]

- ☒ 2: Proc Natl Acad Sci U S A. 2001 Apr 10;98(8):4504-9. Epub 2001 Mar 27.

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Migration of keratinocytes through tunnels of digested fibrin.

Ronfard V, Barrandon Y.

Department of Biology, Ecole Normale Supérieure, 46, Rue d'Ulm 75230 Paris Cedex 05, France.

We report here a hitherto undescribed form of cell migration. When a suspension of human keratinocytes is plated on a fibrin matrix, single cells invade the matrix and progress through it as rounded cells by dissolving the fibrin and thereby creating tunnels. These tunnels are cylindrical or helical, the latter being the result of constant change in the path of cellular advance around the helical axis. Helical tunnel formation is strongly promoted by epidermal growth factor. The rate of migration of the cell through the track of a helical tunnel (up to 2.1 mm per day) is about 7-fold greater than through a cylindrical tunnel. Pericellular fibrinolysis leading to tunnel formation depends on the presence of plasminogen in the medium and its conversion to plasmin by a cellular

activator. Formation of tunnels requires that plasminogen activator be localized on the advancing surface of the keratinocyte; we propose that the tunnel is cylindrical when the site of release of plasmin is located at a fixed point on the cell surface and helical when the site of release precesses.

PMID: 11274362 [PubMed - indexed for MEDLINE]

■ 3: Transplantation. 2000 Dec 15;70(11):1588-98.

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- [Transplantation. 2000 Dec 15;70\(11\):1551-2.](#)

Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix.

Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y.

Departement de Biologie, Ecole Normale Superieure, Paris, France.

BACKGROUND: Extensive third degree burn wounds can be permanently covered by the transplantation of autologous cultured keratinocytes. Many modifications to Green and colleagues' original technique have been suggested, including the use of a fibrin matrix. However, the properties of the cultured cells must be assessed using suitable criteria before a modified method of culture for therapeutic purposes is transferred to clinical use, because changes in culture conditions may reduce keratinocyte lifespan and result in the loss of the transplanted epithelium. **METHODS:** To evaluate the performances of human keratinocytes grown on a fibrin matrix, we assay for their colony-forming ability, their growth potential and their ability to generate an epidermis when grafted onto athymic mice. The results of these experiments allowed us to compare side by side the performance for third degree burn treatment of autologous cultured epithelium grafts grown according to Rheinwald and Green on fibrin matrices with that of grafts grown directly on plastic surfaces. **RESULTS:** We found that human keratinocytes cultured on a fibrin matrix had the same growth capacity and transplantability as those cultured on plastic surfaces and that the presence of a fibrin matrix greatly facilitated the preparation, handling, and surgical transplantation of the grafts, which did not need to be detached enzymatically. The rate of take of grafts grown on fibrin matrices was high, and was similar to that of conventionally cultured grafts. The grafted autologous cells are capable of generating a normal epidermis for many years and favor the regeneration of a superficial dermis. **CONCLUSION:** We have demonstrated that: 1) fibrin matrices have considerable advantages over plastic for the culture of skin cells for grafting and that it is now possible to generate and transplant enough cultured epithelium from a small skin biopsy to restore completely the epidermis of an adult human in 16 days; and 2) the generated epidermis self-renews itself for years. The use of fibrin matrices thus significantly improves the transplantation of cultured epithelium grafts for extensive burns as recently demonstrated in a follow-up work.

PMID: 11152220 [PubMed - indexed for MEDLINE]

■ 4: Burns. 1991 Jun;17(3):181-4.

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Use of human keratinocytes cultured on fibrin glue in the treatment of burn wounds.

Ronfard V, Broly H, Mitchell V, Galizia JP, Hochart D, Chambon E, Pellerin P, Huart JJ.

Blood Transfusion Center of Lille, France.

Keratinocytes isolated from a small skin biopsy and cultured according to the method of Rheinwald and Green (Cell 1975, 6: 331) are able to undergo rapid expansion in vitro and have been used successfully in the treatment of burn wounds. One of the inconveniences of this method involves the transfer of the epidermal sheet from the culture flask onto the wound bed. One way to facilitate this process is to use fibrin glue (Biocol) as a culture bed for the keratinocytes. Burns are then grafted by

simply placing the sheet of fibrin glue and keratinocytes onto the wound bed. This process has been successful in two patients, permanently covering areas of 720 cm² and 5342 cm². The newly formed epidermis was fully differentiated and histologically normal after 1 year. The efficiency of this improved, faster procedure could lead to a new approach in the treatment of extensive burn wounds.

Publication Types:

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PMID: 1892546 [PubMed - indexed for MEDLINE]

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